THE PROSPECTS OF GENE THERAPY FOR MITOCHONDRIAL DISEASES: CAN'T WE DO WITHOUT CRISPR/CAS9?

Chicherin IV[1], Levitsky SA[1], Krasheninnikov IA[1], Tarassov I[2], Kamenski P[1,3] ✉

[1] Faculty of Biology,
Lomonosov Moscow State University, Moscow, Russia

[2] Laboratory of Molecular Genetics, Genomics and Microbiology,
University of Strasbourg, Strasbourg, France

[3] Institute of Living Systems,
Immanuel Kant Baltic Federal University, Kaliningrad, Russia

Mitochondrial DNA mutations cause severe inherited disorders in humans. To date, there are a few therapeutic strategies for their correction; however, it is highly unlikely that they would be routinely used in clinical practice. The past few years have witnessed the rapid progress of a genome editing technology known as CRISPR/Cas9. The present review focuses on the current strategies to combat mitochondrial mutations and reveals their major drawbacks. The article also explores the possibility of creating a possible specific CRISPR/Cas9 tool for correcting mitochondrial DNA mutations and provides a rough description of its mechanism of action. A particular focus is paid to technical challenges. On the whole, we see no principal barriers to implementing a mitoCRISPR/Cas9 system for treating mitochondrial disorders.

Keywords: mitochondrial DNA, mitochondrial diseases, gene therapy, genome editing, CRISPR/Cas9

Funding: Russian Science Foundation, grant No. 14-50-00029 (Lomonosov Moscow State University); *Project 5–100* of the Ministry of Education and Science of the Russian Federation (Immanuel Kant Baltic Federal University); the International Associated Laboratory *RNA-mitocure* (Lomonosov Moscow State University and the University of Strasbourg).

✉ **Correspondence should be addressed:** Peter Kamenski
Leninskie Gory, d. 1, str. 12, Moscow, Russia, 119991 (Faculty of Biology); peter@protein.bio.msu.ru

Received: 20.06.2017 **Accepted:** 23.06.2017

ПЕРСПЕКТИВЫ ГЕННОЙ ТЕРАПИИ МИТОХОНДРИАЛЬНЫХ БОЛЕЗНЕЙ: БЕЗ CRISPR/CAS9 НЕ ОБОЙТИСЬ?

И. В. Чичерин[1], С. А. Левицкий[1], И. А. Крашенинников[1], И. Тарасов[2], П. А. Каменский[1,3] ✉

[1] Биологический факультет,
Московский государственный университет имени М. В. Ломоносова, Москва

[2] Лаборатория молекулярной генетики, геномики и микробиологии,
Страсбургский университет, Страсбург, Франция

[3] Институт живых систем,
Балтийский федеральный университет имени И. Канта, Калининград

Мутации в митохондриальном геноме являются причиной серьезных наследственных заболеваний человека. На сегодняшний день существует несколько способов их коррекции, которые, однако, вряд ли могут быть повсеместно внедрены в клиническую практику. С другой стороны, в последние годы крайне активно развивается технология редактирования геномов CRISPR/Cas9. В работе приводится обзор существующих способов борьбы с митохондриальными мутациями, показываются основные их недостатки. Также анализируются возможности создания версии технологии CRISPR/Cas9 для коррекции мутаций в митохондриальной ДНК, обсуждаются основные этапы, которые необходимо пройти для этого. Особое внимание уделяется техническим сложностям, которые могут возникать при создании такой технологии. В целом принципиальных препятствий к разработке системы mitoCRISPR/Cas9 не выявлено.

Ключевые слова: митохондриальная ДНК, митохондриальные болезни, генная терапия, редактирование геномов, CRISPR/Cas9

Финансирование: Российский научный фонд, грант № 14-50-00029 (МГУ имени М. В. Ломоносова); Программа 5–100 Министерства образования и науки РФ (БФУ им. И. Канта); Международная ассоциированная лаборатория RNA-mitocure (МГУ им. М. В. Ломоносова и Страсбургский университет).

✉ **Для корреспонденции:** Каменский Петр Андреевич
Ленинские горы, д. 1, стр.12, г. Москва, 119991 (биологический факультет); peter@protein.bio.msu.ru

Статья получена: 20.06.2017 **Статья принята к печати:** 23.06.2017

The human mitochondrial genome is a 16.5 kbp-long double-stranded DNA molecule that encodes 13 proteins, 22 tRNAs and rRNAs [1]. Mitochondrial DNA mutations occur in one in about 3,500 people [2]. Due to heteroplasmy (the coexistence of mutant and normal mtDNA copies in the cell), not all such mutations lead to disease even if they affect the coding regions of the mitochondrial genome [3]. Phenotypic expression of mutations always depends on the level of heteroplasmy, i.e. on the ratio of normal to mutant mtDNA molecules. Clinical manifestations of mitochondrial DNA mutations are usually

neuromuscular: nervous and muscular tissues consume large amounts of ATP and are therefore especially vulnerable to mitochondrial dysfunction.

Currently there is no cure for mitochondrial diseases. Treatments the afflicted patients receive aim to merely alleviate their symptoms and only slightly improve the quality of their lives. Over the last two decades, a number of experimental gene-based therapeutic approaches have been developed to suppressing mutations in the mitochondrial genome [4]. Below we highlight some of the main strategies used to combat mitochondrial dysfunction:

– allotopic expression (the gene that encodes a mitochondrial protein of interest is expressed in the cytoplasm; the resulting protein is then imported into the mitochondria and incorporated into the organellar molecular processes instead of the mutant protein);

– xenotopic expression (the orthologous counterpart of the mutant mitochondrial gene taken from another species is expressed in the cytoplasm; the resulting protein is then imported into the mitochondria and incorporated into the organellar molecular processes instead of the mutant protein);

– transfection of human cells by the in vitro synthesized mitochondrial tRNAs or mRNAs, followed by their import into the mitochondria, where they can participate in mitochondrial translation instead of mutant RNAs;

– import into the mitochondria of nucleic acid vectors carrying sequences complementary to the mutant DNA region to which they subsequently bind, thus inhibiting replication of mutant molecules;

– the use of vesicles that can penetrate mitochondrial membranes and thus deliver large biomolecules into the mitochondria where these molecules can maintain normal mitochondrial function.

These approaches have proved to be effective in the experiments on human cell cultures. They share a disadvantage, though: they do not eliminate the mutation itself, but instead reduce the level of heteroplasmy, which in turn leads to partial or full restoration of mitochondrial function. Therefore, even if these approaches were introduced into clinical routine, they would not prevent next generation transmission of mitochondrial mutations. The use of technologies capable of repairing mitochondrial DNA mutations would be a better response to the challenge.

Mitochondrial replacement therapy

It appears that the most effective strategy in combating mutations in the mitochondrial DNA is the so-called Mitochondrial Replacement Therapy (MRT) [5]. It cannot be used to treat mitochondrial disorders in adults, but it does give a female mutation carrier the chance to give birth to a healthy, mutation-free child. In the process of MRT, the diploid nucleus of a fertilized egg received from a female mutation carrier is removed and transferred to an enucleated donor egg received from a healthy woman with normally functional mitochondria. The resulting egg will contain nuclear genetic material donated by the parents and healthy mitochondria from a "second mother". The egg is then implanted into the mother's uterus, and fetal development begins. MRT has already received approval from the UK Parliament for the use in clinics specializing in *in vitro* fertilization. The first "three-parent" child is expected to be born by the end of 2017. However, some countries where such treatments are not subject to legal regulation have already reported the inspiring results of MRT application, namely the birth of healthy children. Mitochondrial replacement therapy is a very convenient and technically simple therapeutic tool, but it raises a lot of ethical questions [6, 7]. In 2016 the US Food and Drug Administration (FDA) started the evaluation process of MRT, but the Congress soon banned this initiative as ethically unacceptable [8]. In view of this, MRT is very unlikely to become a commonly used and widely spread technique.

Zinc-finger endonucleases and mitoTALEN

So far, a few tools for repairing mitochondrial DNA mutations have been engineered based on the molecular technologies for genome editing. These tools raise fewer ethical concerns than MRT. A possibility has been shown to induce sequence-specific double-strand breaks in a mutant DNA molecule using restriction endonucleases [9], zinc-finger nucleases [10] and TALEN enzymes [11]. Breaks in the DNA cause significant shifts in mtDNA heteroplasmy, indicative of linearized molecules degradation. Besides, the mitoTALEN tool has proved to be able to selectively eliminate mutant mitochondrial DNA.

The approaches listed above definitely have the potential to cure human mitochondrial diseases, but they are not perfect. The methods employing restriction endonucleases can be used only for mutations that create the unique recognition site for these enzymes. Of all known deleterious mutations, there is only one capable of doing so. Zinc-finger nucleases and TALENs are more flexible because they can selectively bind to and cut at any DNA region using specially designed protein sequences. Unfortunately, DNA-binding components of these genome editing tools have to be designed and synthesized separately for each mutation. Furthermore, both zinc-finger enzymes and TALENs might turn to be ineffective for repairing certain mutations because their success largely depends on the target DNA sequence. Besides, it has been shown that both technologies have unintended effects on the mitochondrial DNA mediating a decrease in the number of its wildtype copies in the cell [10]. Last, zinc-finger enzymes and TALENs require a particularly careful selection of optimal protein sequences, which is a very labor-intensive, time-consuming and costly procedure.

CRISPR/Cas9

In the light of the above, currently there seems to be no tool for mitochondrial DNA repair that would be effective, technically simple, cheap and ethics-friendly at the same time. However, a technology called CRISPR/Cas9 meets all these requirements.

Its mechanism of action is quite similar to that of TALEN; it consists of a CRISPR guide RNA complementary to a target DNA region and the Cas9 endonuclease that creates a double-strand break in the DNA at the gRNA binding site. CRISPR/Cas9 systems exist in nature in bacteria and are employed by the latter as a defense against bacteriophages. In 2013 CRISPR/Cas9 was successfully used for selective cleavage of human DNA in the living cell [12]. Ever since, the technology has been enjoying a boom of attention because of its immense potential as a therapeutic tool. For example, in 2015 CRISPR/Cas9 was used to repair mutations in the human embryo [13]. It has also found its application in agriculture and is used to obtain new varieties of crops, some of which have already been officially approved for commercial production in a number of countries worldwide [14]. CRISPR/Cas9 is somewhat successfully used for animal genome editing. For example, it was employed to produce tuberculosis-resistant transgenic cattle [15]. However, CRISPR/Cas9-modified animals have not made it yet to the market.

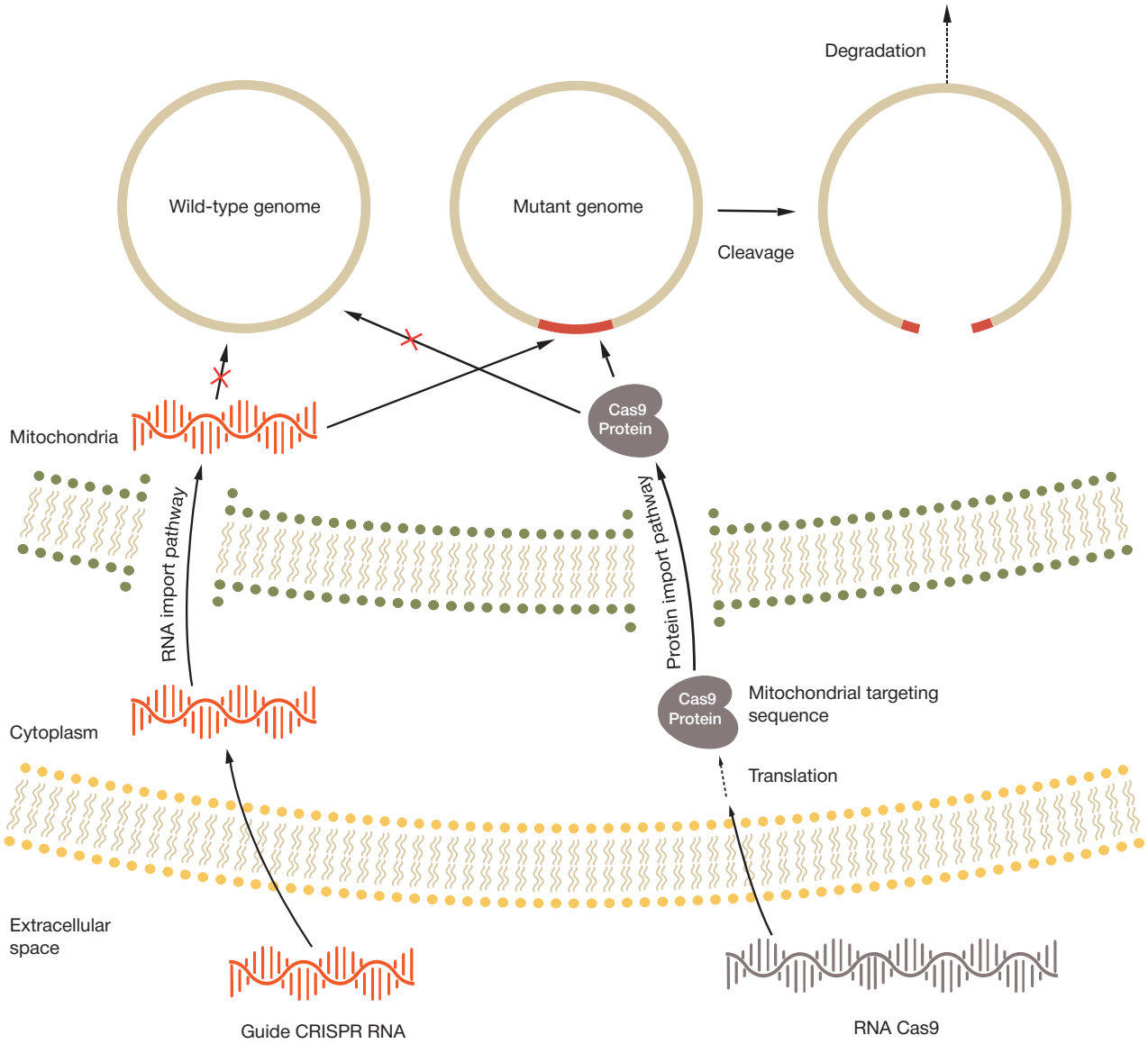
The CRISPR/Cas9 system employs short RNA sequences for targeting a particular genome region, which contributes largely to its popularity since it is not so labor-consuming as zinc fingers or TALEN and certainly cheaper than the latter; it does not require redesigning and synthesizing large protein molecules for every individual mutation. The major drawback of CRISPR/Cas9 is its off-target effect: cleavage of genomic DNA at an off-target site with a sequence similar to that of a target site. Off-target cleavage hardly ever results in the unintended phenotypic changes, but insufficient specificity of the technology poses a serious challenge to researchers worldwide.

Currently a few research teams are attempting to design a CRISPR/Cas9-based platform for mitochondrial DNA editing. Such platform could become a perfect tool for combating deleterious mutations in the mitochondrial genome. First, it would prevent next-generation transmission of mutations by editing human embryos. Second, when combined with targeted delivery systems, CRISPR/Cas9 would help to improve the quality of life of adult people suffering from mitochondrial defects. Third, this platform would be more ethics-friendly than MRT. However, no mitoCRISPR/Cas9 has been created yet. The only description of a potential mitoCRISPR/Cas9 system found in the literature is seriously questioned by the research community due to the low reliability of the research findings.

Creating a mitoCRISPR/Cas9 platform

Below we will briefly describe the main steps to be taken to create a mitoCRISPR/Cas9 platform (see the Figure). We make no claims as to the completeness or correctness of this plan but would like to note that no similar systematic analysis has been carried out so far.

1. *Import of the Cas9 nuclease into mitochondria*. Any eukaryotic cell, including human cells, has a system for protein import into mitochondria that has been very well studied [16]. The majority of proteins that can be imported into these organelles have special signal sequences at their N-termini; these sequences are a few dozen amino acids long, and their presence in the protein is usually a necessary and sufficient condition for its importability. Adding a signal sequence to the N-terminus of a non-imported cytoplasmic protein usually enables its import into the mitochondrion where the signal sequence is cleaved off by mitochondrial proteases and the protein can exhibit its functional activity. There are no obstacles to carrying out a similar procedure with Cas9. Actually, a study



A possible mechanism of action of a hypothetical mitoCRISPR/Cas9 platform

has already been conducted describing a variant of Cas9 that can be imported into human mitochondria [17]. An option of Cas9 gene delivery into the cells includes their transfection by Cas9 mRNA whose translation can be operated by cytoplasmic ribosomes. Provided that the introduced mRNA encodes the mitochondrial signal sequence, the resulting protein will be transported from the cytoplasm into the mitochondrion.

2. *Import of CRISPR guide RNAs to mitochondria and their binding to mutant mitochondrial DNA molecules*. RNA import into mitochondria is known to exist in nature [18]. RNA nucleotide sequences and structural motifs have already been described, that are necessary and sufficient for RNA import into human mitochondria [19, 20]. Besides, it has been shown that chimeric RNAs consisting of such structural motifs and different nucleotide sequences can be effectively delivered to mitochondria and even complementarily bind mtDNA [21]. Chimeric RNAs are also capable of selective binding to mutant DNA; moreover, they can effectively discriminate between point mutations and 'healthy' DNA fragments [22]. To sum up, there is an experimentally confirmed possibility of CRISPR RNA delivery (as part of RNA chimeras) to mitochondria and its selective binding to the mutant regions of the mitochondrial genome.

3. *Preventing CRISPR/Cas9 components from getting into the nucleus*. Technically, there is a chance that CRISPR guide RNAs designed to be imported into mitochondria will end up in the nucleus after transfection, where they can bind to DNA. Adverse effects are unlikely, though, because mitoCas9 cannot enter the nucleus: it does not contain a signal sequence that makes such import possible. Nevertheless, the nucleus should be checked for the presence of Cas9 and CRISPR gRNAs when testing a mitoCRISPR/Cas9 platform.

4. *Cleavage of mutant DNA molecules in mitochondria*. This task should not present a particular difficulty. With all components of the described system delivered to the mitochondrion, the probability of cleavage should remain very high, considering successful performance of CRISPR/Cas9 in the nucleus. In fact, mutant DNA cleavage is possible with the mitoTALEN platform (see above). It should be kept in mind, though, that mtDNA is densely packaged into DNA-protein complexes called nucleoids [23]; their density exceeds that of nuclear DNA. Hypothetically speaking, this circumstance might obstruct DNA cleavage by Cas9. However, considering that (1) no serious problems have been reported for mitoTALEN and (2) dense packaging of mtDNA does not prevent small RNA from binding to it (see above), the nuclease should be able to successfully cut at the mutation site.

5. *Reducing the off-target effect*. This effect is mainly caused by the binding of CRIPSR guide RNAs to off-target genome regions similar to the targeted region in their sequences. The small size of the human mitochondrial genome (~16.5 kbp) renders it highly unlikely. With guide RNAs of a standard size (about 20 nucleotides in length), off-target binding occurs far less frequently than in the nuclear genome. Cleavage and elimination of a few wildtype mitochondrial DNA molecules should not significantly affect the functions of a cell. However, mitoCRISPR/Cas9 should be watched closely for a possible off-target effect.

6. *Elimination of linearized molecules of mutant mitochondrial DNA*. This is the final step. It should not present any particular difficulties, considering that the mitoTALEN tool has already demonstrated the possibility of elimination of linearized molecules (see above). What is more, there is no evidence for the mechanism of mtDNA double strand break repair: linearized DNA molecules are eliminated following break induction [24]. Technically, double strand breaks in mtDNA can initiate recombination involving unaffected molecules. But so far no direct evidence of recombination in human mitochondria has been obtained. Nevertheless, the possibility remains. After Cas9 has exerted its activity, CRISPR guide RNA must for some time remain bound to DNA regions close to the break site. This may entail temporary stabilization of linearized molecules and thus increase the possibility of break repair. Effective mitoCRISPR/Cas9 performance might require temporary inhibition of recombination and repair mechanisms in mitochondria.

CONSLUSION

Considering the current state of knowledge of mitochondrial molecular biology and the advances in CRISPR/Cas9-mediated gene editing, we conclude that there are no insurmountable barriers to creating a variation of the CRISPR/Cas9 tool for mitochondria. We hope that this tool will be designed in the nearest future and become a top approach to suppressing mutations in mitochondrial DNA.

References

1. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J et al. Sequence and organization of the human mitochondrial genome. Nature. 1981 Apr 9; 290 (5806): 457–65.
2. Schaefer AM, Taylor RW, Turnbull DM, Chinnery PF. The epidemiology of mitochondrial disorders — past, present and future. Biochim Biophys Acta. 2004 Dec 6; 1659 (2–3): 115–20.
3. Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. Nat Rev Genet. 2005 May; 6 (5): 389–402.
4. Patrushev MV, Kamenski PA, Mazunin IO. Mutations in mitochondrial DNA and approaches for their correction. Biochemistry (Mosc). 2014 Nov; 79 (11): 1151–60. DOI: 10.1134/S0006297914110029.
5. Wolf DP, Mitalipov N, Mitalipov S. Mitochondrial replacement therapy in reproductive medicine. Trends Mol Med. 2015 Feb; 21 (2): 68–76. DOI: 10.1016/j.molmed.2014.12.001.
6. Rulli T. The Mitochondrial Replacement 'Therapy' Myth. Bioethics. 2017 Jun; 31 (5): 368–74. DOI: 10.1111/bioe.12332.
7. Burrell C. Mitochondrial replacement therapy and 'three-parent children' — who should be registered as the legal parents? BJOG. 2017 Jun; 124 (7): 1056. DOI: 10.1111/1471-0528.14638.
8. Adashi EY, Cohen IG. Mitochondrial Replacement Therapy: Unmade in the USA. JAMA. 2017 Feb 14; 317 (6): 574–5. DOI: 10.1001/jama.2016.20935.
9. Tanaka M, Borgeld HJ, Zhang J, Muramatsu S, Gong JS, Yoneda M et al. Gene therapy for mitochondrial disease by delivering restriction endonuclease SmaI into mitochondria. J Biomed Sci. 2002; 9 (6 Pt 1): 534–41.
10. Gammage PA, Gaude E, Van Haute L, Rebelo-Guiomar P, Jackson CB, Rorbach J, et al. Near-complete elimination of mutant mtDNA by iterative or dynamic dose-controlled treatment with mtZFNs. Nucleic Acids Res. 2016 Sep 19; 44 (16): 7804–16. DOI: 10.1093/nar/gkw676.
11. Bacman SR, Williams SL, Pinto M, Peralta S, Moraes CT. Specific elimination of mutant mitochondrial genomes in patient-derived cells by mitoTALENs. Nat Med. 2013 Sep; 19 (9): 1111–3. DOI: 10.1038/nm.3261.
12. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014 Nov 28; 346 (6213): 1258096. DOI: 10.1126/science.1258096.
13. Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z et al. CRISPR/

Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell. 2015 May; 6 (5): 363–72. DOI: 10.1007/s13238-015-0153-5.
14. Belhaj K, Chaparro-Garcia A, Kamoun S, Patron NJ, Nekrasov V. Editing plant genomes with CRISPR/Cas9. Curr Opin Biotechnol. 2015 Apr; 32: 76–84. DOI: 10.1016/j.copbio.2014.11.007.
15. Gao Y, Wu H, Wang Y, Liu X, Chen L, Li Q et al. Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects. Genome Biol. 2017 Feb 1;18 (1): 13. DOI: 10.1186/s13059-016-1144-4.
16. Straub SP, Stiller SB, Wiedemann N, Pfanner N. Dynamic organization of the mitochondrial protein import machinery. Biol Chem. 2016 Nov 1; 397 (11): 1097–114. DOI: 10.1515/hsz-2016-0145.
17. Orishchenko KE, Sofronova JK, Chupakhin EG, Lunev EA, Mazunin IO. Delivery Cas9 into mitochondria. Genes and Cells. 2016; 11: 100–5.
18. Sieber F, Duchene AM, Marechal-Drouard L. Mitochondrial RNA import: from diversity of natural mechanisms to potential applications. Int Rev Cell Mol Biol. 2011; 287: 145–90. DOI: 10.1016/B978-0-12-386043-9.00004-9.
19. Wang G, Shimada E, Zhang J, Hong JS, Smith GM, Teitell MA et al. Correcting human mitochondrial mutations with targeted RNA import. Proc Natl Acad Sci U S A. 2012 Mar 27; 109 (13): 4840–5. DOI: 10.1073/pnas.1116792109.
20. Kolesnikova O, Kazakova H, Comte C, Steinberg S, Kamenski P, Martin RP et al. Selection of RNA aptamers imported into yeast and human mitochondria. RNA. 2010 May; 16 (5): 926–41. DOI: 10.1261/rna.1914110.
21. Comte C, Tonin Y, Heckel-Mager AM, Boucheham A, Smirnov A, Aure K, et al. Mitochondrial targeting of recombinant RNAs modulates the level of a heteroplasmic mutation in human mitochondrial DNA associated with Kearns Sayre Syndrome. Nucleic Acids Res. 2013 Jan 7; 41 (1): 418–33. DOI: 10.1093/nar/gks965.
22. Tonin Y, Heckel AM, Vysokikh M, Dovydenko I, Meschaninova M, Rotig A, et al. Modeling of antigenomic therapy of mitochondrial diseases by mitochondrially addressed RNA targeting a pathogenic point mutation in mitochondrial DNA. J Biol Chem. 2014 May 9; 289 (19): 13323–34. DOI: 10.1074/jbc.M113.528968.
23. Kolesnikov AA. The Mitochondrial Genome. The Nucleoid, Biochemistry (Mosc). 2016; 81 (2): 1057–65.
24. Moretton A, Morel F, Macao B, Lachaume P, Ishak L, Lefebvre M et al. Selective mitochondrial DNA degradation following double-strand breaks. PLoS One. 2017 Apr 28; 12 (4): e0176795. DOI: 10.1371/journal.pone.0176795.

Литература

1. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J et al. Sequence and organization of the human mitochondrial genome. Nature. 1981 Apr 9; 290 (5806): 457–65.
2. Schaefer AM, Taylor RW, Turnbull DM, Chinnery PF. The epidemiology of mitochondrial disorders — past, present and future. Biochim Biophys Acta. 2004 Dec 6; 1659 (2–3): 115–20.
3. Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. Nat Rev Genet. 2005 May; 6 (5): 389–402.
4. Patrushev MV, Kamenski PA, Mazunin IO. Mutations in mitochondrial DNA and approaches for their correction. Biochemistry (Mosc). 2014 Nov; 79 (11): 1151–60. DOI: 10.1134/S0006297914110029.
5. Wolf DP, Mitalipov N, Mitalipov S. Mitochondrial replacement therapy in reproductive medicine. Trends Mol Med. 2015 Feb; 21 (2): 68–76. DOI: 10.1016/j.molmed.2014.12.001.
6. Rulli T. The Mitochondrial Replacement 'Therapy' Myth. Bioethics. 2017 Jun; 31 (5): 368–74. DOI: 10.1111/bioe.12332.
7. Burrell C. Mitochondrial replacement therapy and 'three-parent children' — who should be registered as the legal parents? BJOG. 2017 Jun; 124 (7): 1056. DOI: 10.1111/1471-0528.14638.
8. Adashi EY, Cohen IG. Mitochondrial Replacement Therapy: Unmade in the USA. JAMA. 2017 Feb 14; 317 (6): 574–5. DOI: 10.1001/jama.2016.20935.
9. Tanaka M, Borgeld HJ, Zhang J, Muramatsu S, Gong JS, Yoneda M et al. Gene therapy for mitochondrial disease by delivering restriction endonuclease SmaI into mitochondria. J Biomed Sci. 2002; 9 (6 Pt 1): 534–41.
10. Gammage PA, Gaude E, Van Haute L, Rebelo-Guiomar P, Jackson CB, Rorbach J et al. Near-complete elimination of mutant mtDNA by iterative or dynamic dose-controlled treatment with mtZFNs. Nucleic Acids Res. 2016 Sep 19; 44 (16): 7804–16. DOI: 10.1093/nar/gkw676.
11. Bacman SR, Williams SL, Pinto M, Peralta S, Moraes CT. Specific elimination of mutant mitochondrial genomes in patient-derived cells by mitoTALENs. Nat Med. 2013 Sep; 19 (9): 1111–3. DOI: 10.1038/nm.3261.
12. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014 Nov 28; 346 (6213): 1258096. DOI: 10.1126/science.1258096.
13. Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z et al. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell. 2015 May; 6 (5): 363–72. DOI: 10.1007/s13238-015-0153-5.
14. Belhaj K, Chaparro-Garcia A, Kamoun S, Patron NJ, Nekrasov V. Editing plant genomes with CRISPR/Cas9. Curr Opin Biotechnol. 2015 Apr; 32: 76–84. DOI: 10.1016/j.copbio.2014.11.007.
15. Gao Y, Wu H, Wang Y, Liu X, Chen L, Li Q et al. Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects. Genome Biol. 2017 Feb 1;18 (1): 13. DOI: 10.1186/s13059-016-1144-4.
16. Straub SP, Stiller SB, Wiedemann N, Pfanner N. Dynamic organization of the mitochondrial protein import machinery. Biol Chem. 2016 Nov 1; 397 (11): 1097–114. DOI: 10.1515/hsz-2016-0145.
17. Orishchenko KE, Sofronova JK, Chupakhin EG, Lunev EA, Mazunin IO. Delivery Cas9 into mitochondria. Genes and Cells. 2016; 11: 100–5.
18. Sieber F, Duchene AM, Marechal-Drouard L. Mitochondrial RNA import: from diversity of natural mechanisms to potential applications. Int Rev Cell Mol Biol. 2011; 287: 145–90. DOI: 10.1016/B978-0-12-386043-9.00004-9.
19. Wang G, Shimada E, Zhang J, Hong JS, Smith GM, Teitell MA et al. Correcting human mitochondrial mutations with targeted RNA import. Proc Natl Acad Sci U S A. 2012 Mar 27; 109 (13): 4840–5. DOI: 10.1073/pnas.1116792109.
20. Kolesnikova O, Kazakova H, Comte C, Steinberg S, Kamenski P, Martin RP et al. Selection of RNA aptamers imported into yeast and human mitochondria. RNA. 2010 May; 16 (5): 926–41. DOI: 10.1261/rna.1914110.
21. Comte C, Tonin Y, Heckel-Mager AM, Boucheham A, Smirnov A, Aure K et al. Mitochondrial targeting of recombinant RNAs modulates the level of a heteroplasmic mutation in human mitochondrial DNA associated with Kearns Sayre Syndrome. Nucleic Acids Res. 2013 Jan 7; 41 (1): 418–33. DOI: 10.1093/nar/gks965.
22. Tonin Y, Heckel AM, Vysokikh M, Dovydenko I, Meschaninova M, Rotig A et al. Modeling of antigenomic therapy of mitochondrial diseases by mitochondrially addressed RNA targeting a pathogenic point mutation in mitochondrial DNA. J Biol Chem. 2014 May 9; 289 (19): 13323–34. DOI: 10.1074/jbc.M113.528968.
23. Kolesnikov AA. The Mitochondrial Genome. The Nucleoid, Biochemistry (Mosc). 2016; 81 (2): 1057–65.
24. Moretton A, Morel F, Macao B, Lachaume P, Ishak L, Lefebvre M et al. Selective mitochondrial DNA degradation following double-strand breaks. PLoS One. 2017 Apr 28; 12 (4): e0176795. DOI: 10.1371/journal.pone.0176795.